mids belonging to the same group isolated from strains which are epidemiologically related confirm that they possess large sequences in common. Some groups of plasmids can be considered as 'species'.
It is possible today to classify the main groups which have been identified into several naturally occurring strains belonging to different bacterial genera and isolated in different geographical sites according to their DNA homology. 80% of 150 plasmids from gram-negative rods examined by us belong to the following groups:
1. Groups with large homology with respect to transfer operon and pili formation but subdivided by incompatibility phenomenon. F like plasmids: Inc F, FII, FIII. I like plasmids: IncI1, IncI2.
2. Groups of incomptatible plasmids with poor homology. Complex Inc H: H1, H2, H3. Complex Inc Y: Y1, Y2.
3. Independent groups. - Localisation of R determinants in a few areas (?): Inc M, Inc C. - Several insertions (?): Inc P, Inc N, Inc W. - Others: Inc B.
Assuming that plasmids belonging to a given Inc group are composed of a relatively stable 'core' into which several transposons can be inserted at various preferential sites, one may expect important differences among epidemiologically unrelated plasmids but the maintenance of an identical structure in an epidemic plasmid.

A - Unrelated Inc I1 plasmids

By restriction endonuclease analysis, 5 unrelated plasmids belonging to Inc I1 group share 15 *EcoR1* fragments in common.
The technique described by Southern to detect partial or complete homology between the DNA fragments has been used. In vitro ^{32}P-labelled complementary RNA from pIP111, a 'transfer factor' with no detectable R-determinant, was used as a probe. The autoradiogram shows hybridization with the 15 fragments common to the five plasmids studied (P9, pIP186, pIP112, pIP565 and pIP111).
Electron microscopy of heteroduplex between the transfer factor pIP111 and others shows a unique single stranded DNA insertion loop to be always located at the same distance from a small region with impaired sequence.
A restriction endonuclease map of the inserted regions shows differences between the size and the location of fragments corresponding to the resistance characters. Thus, by means of these analyes it is possible to differentiate between these unrelated plasmids in spite of their large homology.

B - Epidemiologically related plasmids

A plasmid belonging to incompatibility group C coding for gentamicin resistance by an adenylating enzyme and for ampicillin resistance by a peculiar oxacillin hydrolysing β-lactamase, was observed by J. Witchitz at the Claude-Bernard hospital in Paris among 12 different bacterial species, including *Pseudomonas aeruginosa*, between November 1969 and December 1975. DNA/DNA hybridization by A. Roussel showed a high degree of homology between plasmids isolated from these different bacterial species at different period of time.
Analysis after digestion with *EcoR1* and agarose gel electrophoresis showed very few differences among the 10 fragments generated by plasmids isolated in 1969 and 1975. These data indicate a high degree of structural stability among the Inc C plasmids through numerous cycles of replication and transfer in vivo.

In conclusion, when the structure of a plasmid has been almost completely investigated using the sophisticated techniques of molecular biology it is possible to differentiate between epidemiologically related and unrelated plasmids. But simplifications of these techniques are needed which can be easily adopted for real epidemiological surveys.

Antimicrobial chemotherapy - a clinician's viewpoint*

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Antimicrobial chemotherapy requires a sound knowledge of clinical microbiology, infectious diseases and the pharmacology of antibiotics. Two examples should illustrate the point that usage of antimicrobial agents in Switzerland is far from optimal.
In the first 3 months of 1979, we conducted a quality-of-use study of antibiotic in a surgical clinic in Zürich[10]. A record was kept of every patient treated with an antibiotic, listing the symptoms, the choice of antibiotics, the dosage and duration of therapy, side-effects noted, and the costs of medication, as well as all the available clinical, microbiological and laboratory data. Each treatment course was assessed and divided in to 3 categories according to the criteria of

Kunin et al.[3] (table 1). Table 2 contains an analysis of 178 prophylactic and therapeutic courses and shows the total costs of therapy in each of these categories. These figures demonstrate that none of the prophylactic courses was carried out correctly. The most common errors in category II were the inappropriate selection of the antibiotic (53%), inadequate duration of therapy (16%), or inadequate dosage (12%). Frequent errors in category III included the prophylactic use of antibiotics in patients with indwelling urinary catheters (35%) or the treatment of asymptomatic bacteruria (16%). Altogether 35% of the antibiotic therapies were unnecessary, and in more than 40% of the cases patients would have profited from a more appropriate choice or use of the antibiotic.

The 2nd example concerns the use of antibiotics in general practice. Since no detailed study of this aspect has yet been made in Switzerland, only tentative conclusions can be drawn from amounts of antibiotics prescribed yearly by doctors in private practice. Nevertheless one single example may suffice to show that there is much room for improvement. In 1978, almost 600,000 Swiss-francs-worth of oral tetracycline suspension was sold in Switzerland on prescription. This is equivalent to about 28 kg, or 246,000 days of therapy. Not included in these figures are tetracycline suspensions dispensed directly by doctors. Conservative estimates of these prescriptions are in a similar order of magnitude. Tetracycline suspensions are administered almost exclusively to small children. However, according to opinions of leading pediatricians, there is no indication for tetracycline therapy in children and therefore this type of therapy falls in category II (inappropriate choice and/or use of antibiotic). Ray and co-workers[23], who made a survey of the administration of tetracyclines to children in Tennessee, reported similar results. The same authors investigated the use of chloramphenicol by general practitioners and likewise showed that in the majority of cases chloramphenicol therapy was not indicated[22]. These 2 examples very clearly reveal the need for improvement in the teaching of medical students and in the continuing education of physicians in the field of chemotherapy and infectious diseases.

In this paper, some practical solutions are suggested in which clinical microbiologists could help to improve the usage of antimicrobial agents through their everyday contacts with physicians. These relate to the suitability of specimens for bacteriological examination, and to the efficiency of microbiological laboratory services, which, in turn may influence the choice and usage of antibiotics.

Table 1. Criteria for antimicrobial chemotherapy

Category I	Indication correct; appropriate choice and use of antibiotic.
Category II	Indication correct; inappropriate choice and/or use of antibiotic.
Category III	Administration of an antibiotic not indicated.

Table 2. Use of antibiotics in a surgical clinic

Category	I	II	III	Total
Prophylaxis	0	35	48	83
Therapy	39	41	15	95
Costs (SFr.)	4400	9600	2400	16,000

178 antibiotic treatment courses in 154 patients.

Suitability of specimens for bacteriological examination

The bacterial examination of saliva instead of sputum is timeconsuming and leads to erroneous conclusions. Specimens submitted for bacterial culture should be examined microscopically to determine whether it is sputum or saliva. Only specimens containing more than 25 leucocytes and fewer than 10 squamous epithelial cells per microscopic field (100 times magnification) should be processed. If these criteria are not met, the sender should be informed immediately. The written report should contain a brief statement such as: 'Microscopic examination revealed < 25 leucocytes and > 10 epithelial cells per field, indicating oropharyngeal contamination. Please repeat the sputum examination'[19,28]. This procedure could result in a definite improvement of bacterial sputum cultures and may be compared with the introduction of dipslide cultures for the diagnosis of urinary tract infections, which after a considerable period of adaptation, are now generally accepted as the method of first choice.

A similar problem arises in the microbiological diagnosis of chronic osteomyelitis. The diagnostic value of sinus tract cultures in cases of chronic osteomyelitis has been examined by Mackowiak et al.[18]. 183 preoperative sinus tract cultures were compared with cultures of operative specimens in 35 patients in which a single pathogen was isolated from the operation site (*Staphylococcus aureus,* n=21; Enterobacteriaceae, n=8; *Pseudomonas aeruginosa,* n=3; *Streptococcus* sp., n=3). In only 40/183 cultures the sinus tract culture contained the operative pathogen in pure culture (usually *S. aureus*). In 8/183 the preponderant organism from the sinus tract coincided with the operative pathogen, in 33/183 the operative pathogen was found in a mixed sinus tract culture and in 102/183 the operative pathogen could not be recovered from the sinus tract. The authors concluded that a bacteriologic diagnosis of chronic osteomyelitis based on isolation of common pathogens other than *S. aureus* from sinus tract must be verified by an appropriate operative culture.

Experience over the past few years has shown that anaerobic bacteria play a predominant role in infec-

tions of the peritoneal cavity and brain abcesses. Discrepancies between the results of microscopic examination of gram-stained smears and cultures should alert microbiologists that specimens were transported under unsuitable, i.e. aerobic conditions. This problem can easily be solved if the microbiological institutes supply practicing physicians and clinicians with anaerobic transportation media.

The same applies to the diagnosis of gonorrhoea in women. A diagnosis based on a gram-stain is unreliable because of a low sensitivity and specificity. Endocervical and urethral cultures are absolutely necessary but require appropriate transportation media.

These 4 examples illustrate where improvements could be easily achieved. A direct interaction between physicians and microbiologists is undoubtedly more productive than seminars and lectures. Furthermore, a phone call is less time-consuming than the isolation, identification and sensitivity testing of several different species from a buccal flora.

Improvement of the services provided by microbiological laboratories

Frequently antimicrobial chemotherapy needs individual tailoring to the patients' specific conditions. However, shortcomings in the existing diagnostic facilities and lack of personnel are limiting factors, especially in a small microbiological laboratory. Nevertheless the following 3 aspects deserve particular attention, since institution or improvement of these services will ultimately result in better patient care.

1. Rapid identification of bacteria and fungi from blood and cerebro-spinal fluid

This would be possible if emergency services were provided during the night and at week-ends. 2ndly wider use of new culture techniques such as the $C^{14}O_2$-test, could shorten the time needed for bacteriological examinations and possibly improve their sensitivity.

The present-day economic situation requires hospital administrators to weigh the financial aspects of semiautomatic systems (such as Bactec®) very carefully against the benefits derived from such a system. But how should financial savings, accomplished by earlier institution of specific chemotherapy be evaluated? The centrifugation technique for blood cultures is another example of a rapid method with requires less equipment and may be more sensitive than conventional methods[7,8].

2. Differentiated sensitivity tests

The agar diffusion test according to Kirby-Bauer or the ICS-method is entirely adequate to select the appropriate antibiotic for infections with a benign clinical course. However, for the effective treatment of infective endocarditis, quantitative data in the form of MIC and MBC values are absolutely indispensable[9]. In patients with septicemia, particularly in the compromised host, various authors have shown a good correlation between the MIC and antibiotic serum levels on the one hand, and the clinical outcome on the other. This relation between dosage and efficacy is especially well documented for the aminoglycoside antibiotics[1,5,11,13,20,27]. Aminoglycosides and amphotericin B, which are essential agents in the chemotherapy of meningitis due to gram-negative bacteria or fungi, penetrate only poorly into the CSF despite the presence of meningeal inflammation. Knowledge of the MIC's enables the clinician to be more discriminative in choosing the appropriate agent and adjusting the dosage.

Osteomyelitis and bacterial arthritis are infectious diseases that require antibiotic therapy lasting for several weeks. Even if unsuccessful therapy may not have such grave consequences as in meningitis, residual function is often seriously impaired. In a considerable number of patients the antibiotic has to be changed because of severe side-effects or because prolonged i.v. administration is not feasible. In such cases, too, the patient's chances of deriving the maximum benefit are much greater if quantitative information on the sensitivity of the causative organism is available[6].

In life-threatening systemic infections, particularly in neutropenic patients, various combinations of a β-lactam and an aminoglycoside antibiotic, have been shown to produce significantly better therapeutic results[2,21]. Although the checker-board method of determining a synergistic antibiotic combination is time-consuming, it does help the clinician considerably in making his decision. The development of micromethods (e.g. the Microtiter System®) has greatly reduced the work involved for quantitative estimations (MIC, MBC, synergy testing), which should justify the introduction of such methods even in small laboratories.

Periodical information on prevailing sensitivity profiles within a hospital or unit (e.g. intensive care unit) for frequently isolated pathogens and commonly used antibiotics is another useful service which can be provided by microbiological laboratories[15,25]. When the patient's previous medical history and the clinical situation are taken into account, the spectrum of potential pathogens may be narrowed with a fair degree of certainty to a few likely species. Knowledge of the resistance profile within this specific setting enables the physician to make an 'intelligent guess' at the right antibiotic before the causative organism is known or a standard disc sensitivity test has been performed.

3. Monitoring of antimicrobial chemotherapy

The efficacy of treatment can be checked either by testing the bactericidal activity of the patient's serum against the offending pathogen or by periodical monitoring of the antibiotic concentrations in the serum. Several authors advocate the former test as a mean of ensuring optimal efficacy in the treatment of endocarditis and septicemia[16,26]. The major disadvantage of this method is the lack of a standardized technique. Furthermore only the treatment of culture-positive infections can be assessed.

Periodical measurements of serum concentrations are of greater practical importance, particularly in patients receiving aminoglycosides. In vitro and in vivo studies demonstrate a quantitative relationship between aminoglycoside concentrations in the blood and their clinical efficacy[1,5,11,13,20,27]. By contrast, the relationship between administered doses of aminoglycosides and attainable serum concentrations is fairly unpredictable[4,12,14,17,24,29]. Therefore it appears mandatory to monitor aminoglycoside serum concentrations in critical situations such as life-threatening infections or in patients with unstable renal function. Determinations of serum levels of non-aminoglycoside antibiotics may be useful in dialysis patients or to test compliance in unreliable patients with tuberculosis.

In conclusion, continuous education of physicians – provided by clinical microbiologists – is essential to ensure optimal antimicrobial chemotherapy. The importance of determining the suitability of specimens submitted to the microbiological laboratories and the need for greater efficiency in the service facilities are discussed as examples of how the microbiologist can best utilize his knowledge of infectious diseases and the pharmacology of antibiotics to the benefit of the patients.

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1 E.T. Anderson, L.S. Young and W.L. Hewitt, Am. J. Med. *61*, 493–497 (1976).
2 E.T. Anderson, L.S. Young and W.L. Hewitt, Chemotherapy *24*, 45–54 (1978).
3 Audits of antimicrobial usage. Guidelines for peer review. J. Am. med. Ass. *237*, 1001–1008, 1134–1137, 1241–1245, 1366–1369, 1481–1484, 1605–1608, 1723–1725, 1859–1860, 1967–1970 (1977).
4 M. Barza, B.R. Brown, D. Shen, M. Gibaldi and L. Weinstein, J. infect. Dis. *132*, 165–174 (1975).
5 G.P. Bodey, V. Rodriguez, M. Valdivieso and R. Feld, J. infect. Dis. *134*, Suppl., 421–427 (1976).
6 J.L. Bussière, R. Lopitaux, J. Sirot, R. Cluzel and S. Rampon, Revue Rhum. Mal, osteo-artic. *45*, 259–268 (1978).
7 G.L. Dorn and K. Smith, J. clin. Microbiol. *7*, 52–54 (1978).
8 G.L. Dorn, G.A. Land and G.E. Wilson, J. clin. Microbiol. *9*, 391–396 (1979).
9 R. Eckhardt, R. Lüthy and W. Siegenthaler, in: Current Chemotherapy, p. 268–269. Ed. W. Siegenthaler and R. Lüthy. Am. Soc. Microbiol., Washington DC 1978.
10 A. Eijsten, R. Lüthy and A. Akovbiantz, Schweiz. Med. Wschr. *109*, 1931–1936 (1979).
11 R. Feld, M. Valdivieso, G.P. Bodey and V. Rodriguez, Am. J. med. Sci. *274*, 179–188 (1977).
12 E.L. Goodman, J. Van Gelder, R. Holmes, A.R. Hull and J.P. Sanford, Antimicrob. Agents Chemother. *8*, 434–438 (1975).
13 G.G. Jackson and L.J. Riff, J. infect. Dis. *124*, Suppl., 185–191 (1971).
14 D. Kaye, M.E. Levison and E.D. Labovitz, J. infect. Dis. *130*, 150–154 (1974).
15 F.H. Kayser, J. Wüst and J. Munzinger, Schweiz. Rundschau Med. (Praxis) *66*, 669–675 (1977).
16 J. Klastersky, D. Daneau, G. Swings and D. Weerts, J. infect. Dis. *129*, 187–193 (1974).
17 R. Lüthy, Infection *7*, in press (1979).
18 P.A. Mackowiack, S.R. Jones and J.W. Smith, J. Am. med. Ass. *239*, 2772–2775 (1978).
19 P.R. Murray and J.A. Washington, Mayo Clinic Proc. *50*, 339–344 (1975).
20 P. Noone, T.M. Parsons, J.R. Pattison, R.C.B. Slack, D. Garfield-Davies and K. Hughes, Br. med. J. *1*, 477–481 (1974).
21 J.J. Rahal, Medicine *57*, 179–195 (1978).
22 W.A. Ray, C.F. Federspiel and W. Schaffner, Ann. intern. Med. *84*, 266–270 (1976).
23 W.A. Ray, C.F. Federspiel and W. Schaffner, J. Am. med. Ass. *237*, 2069–2074 (1977).
24 L.J. Riff and G.G. Jackson, J. infect. Dis. *124*, Suppl., 98–105 (1971).
25 A. Roupas and J.D. Piguet, Méd. Hyg. (Genève) *34*, 989–1000 (1976).
26 J.G. Schlichter and H. MacLean, Am. Heart J. *34*, 209–211 (1947).
27 M. Valdivieso, R. Feld, V. Rodriguez and G.P. Bodey, Am. J. med. Sci. *270*, 453–463 (1975).
28 R.E. Van Scoy, Mayo Clin. Proc. *52*, 39–45 (1977).
29 R.E. Winters, K.D. Litwack and W.L. Hewitt, J. infect. Dis. *124*, Suppl, 90–95 (1971).

Scope and limitations of experimental chemotherapy*

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The beginnings of experimental chemotherapy, which might best be defined as 'the treatment of animals with simulated human infections', date back to the turn of the century[15,40]. Since then, it has evolved to become an indispensable tool for research into the treatment of infectious diseases and for antibiotic research in particular. This is clearly evident from the annual crop of publications on the subject. Of 519